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CROSSBREEDING BETWEEN CLEARFIELD® RICE WITH WEEDY RICE UNDER VARIOUS CONDITIONS

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By

ENGKU AHMAD KHAIRI BIN ENGKU ARIFF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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February 2016

Chairman : Norida Mazlan, PhD
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Rice is an important crop in our country as it is our staple food. Due to huge productivity losses because of weeds, the imidazolinone-resistant Clearfield® rice was developed to control it. Its close genetic relation with the weedy rice makes it a good candidate for hybridization, producing super weeds. The main objective of this study was to determine whether gene flow from Clearfield® rice to weedy rice can occur. This study has three experiments. In the first experiment, Clearfield® rice varieties (CL1, CL2) and weedy rice variants (V1, V2, V3, V4) were planted to observe the morphological characteristics. The second experiment was conducted in rice field for two seasons. The first (dry) season, two variants of weedy rice (V1, V2) and four variants (V1, V2, V3, V4) were used in and the second (rainy) season and were planted at a distance of 1m, 2m, 3m 4m and 5m from the Clearfield® rice. Seeds (F1) from weedy rice were collected and germinated in trays before were sprayed with OnDuty™ at day fourteen with a rate of 220 g/ha. The third experiment was determining hybrids using Simple Sequence Repeat (SSR) primer RM251 using leaves for the DNA extraction. The first experiment showed that weedy rice was morphologically superior to Clearfield® rice whereby it had double the number of tillers (more than 30) and almost 50 cm taller. In the second study after spraying OnDuty™, CL2 has significant difference at 20.38% compared to CL1 at 13.00% in second season. V1 showed the highest survival percentage, at 11.15% and 22.45% in both season. CL2 and V2 were the best combination of parent with 28.91% seedlings survived. About 80% seedlings survived from CL2V1 at the distance of 1m in second season. Higher number of overlapping period and wind speed in the second season were considered to affect survival percentages. The third study shows that molecular analysis has determined seven hybrids among the seedlings using primer RM251 with hybrids producing three bands. In conclusion, Clearfield® rice can hybridize with weedy rice under field condition and the percentages could increase with days of overlapping and wind speed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

KACUKAN ANTARA PADI CLEARFIELD®
DENGAN PADI ANGIN DI BAWAH PELBAGAI KEADAAN

Oleh

ENGKU AHMAD KHAIRI BIN ENGKU ARIFF

Februari 2016

Pengerusi : Norida Mazlan, PhD
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Padi merupakan tanaman penting di negara kita kerana ia adalah makanan ruji. Oleh kerana kerugian produktiviti yang banyak disebabkan rumpai, padi Clearfield® yang rintang imidazolinone telah dibangunkan untuk mengawalnya. Genetiknya yang berkait rapat dengan padi angin menjadikannya calon yang baik untuk penghibridan, menghasilkan rumpai super. Objektif utama kajian ini adalah untuk menentukan sama ada aliran gen daripada padi Clearfield® ke padi angin boleh berlaku. Kajian ini mempunyai tiga eksperimen. Dalam eksperimen pertama, varieti padi Clearfield® (CL1, CL2) dan varian padi angin (V1, V2, V3, V4) telah ditanam untuk melihat ciri-ciri morfologinya. Eksperimen kedua dijalankan di sawah untuk dua musim. Musim pertama (kering), dua varian (V1, V2) digunakan dan empat varian padi angin (V1, V2, V3, V4) telah digunakan dalam musim kedua (hujan) dan telah ditanam pada jarak 1m, 2m, 3m 4m dan 5m daripada padi Clearfield®. Benih (F1) dari padi angin telah dikumpulkan dan dicambahkan dalam dulang sebelum disembur dengan OnDuty ™ pada hari empat belas dengan kadar 220 g / ha. Eksperimen ketiga adalah menentukan hibrid menggunakan primer Ulang Urutan Mudah (SSR) RM251 menggunakan daun untuk pengekstrakan DNA. Eksperimen pertama menunjukkan bahawa padi angin mempunyai morfologi lebih baik daripada padi Clearfield® di mana ia mempunyai dua kali ganda bilangan anak padi (lebih daripada 30) dan hampir 50 cm lebih tinggi. Dalam kajian kedua selepas menyembur OnDuty ™, CL2 mempunyai perbezaan yang signifikan pada 20.38% berbanding dengan CL1 pada 13.00% pada musim kedua. V1 menunjukkan peratusan hidup yang paling tinggi, pada 11.15% dan 22.45% dalam kedua-dua musim. CL2 dan V2 adalah kombinasi terbaik induk padi dengan 28.91% benih terselamat. Kira-kira 80% benih terselamat dari CL2V1 pada jarak 1m dalam musim kedua. Tempoh bertindihan dan kelajuan angin yang lebih tinggi pada musim kedua telah dianggap mempengaruhi peratusan hidup. Kajian ketiga menunjukkan bahawa analisis molekul telah mengenalpasti tujuh kacukan daripada anak benih

menggunakan primer RM251 dengan kacukan menghasilkan tiga jalur. Kesimpulannya, padi Clearfield® boleh kacuk silang dengan padi angin di bawah keadaan lapangan dan kadar itu boleh meningkat disebabkan hari pertindihan dan kelajuan angin.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by graduate student

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This is to confirm that:

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LIST OF ABBREVIATIONS

®	Registered trademark
™	Trademark
µ	micro
ALS	acetolactate synthase
ANOVA	Analysis of variance
Bt	*Bacillus thuringiensis*
DAS	Day after seeding
DAT	Day after transplanting
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
g	Gram
ha	Hectare
ht	Height
IGMORIS	Indian GMO research Information system
IMI	Imidazolinone
IRRI	International Rice Research Institute
ISAAA	International service for the Acquisition of Agri-biotech Application
L	Litre
LKPP	Lembaga Kemajuan Perusahaan Pertanian
m	metre
MARDI	Malaysian Agriculture Research and Development Institute
min	Minute
mm	millimeter
MRL	Maximum Residue Limit

mt	Million tons
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
Sdn Bhd	Sendirian Berhad
SSR	Simple sequence primer
SSLP	simple sequence length polymorphism
TAE	Tris/acetate/ Ethylenediaminetetraacetic acid
TBE	Tris/Borate/Ethylenediaminetetraacetic acid
Ti	Tillering ability
USD	United States Dollar

CHAPTER 1

INTRODUCTION

Rice is a very important crop in Asia as 90% of all rice production are consumed in this region (Gealy *et al.*, 2003). It is the main staple food in Malaysia and the third largest crop production after palm oil and rubber. However rice is very weak in term of competitiveness as yield loss caused by weeds can go up to 35% (Karim *et al.*, 2004). Under field conditions, weedy rice can absorb up to 60% of nitrogen (N) fertilizer that was applied (Burgos *et al,* 2006). Most of the weeds that can cause serious economic problems are the wild *Oryza*. They compete for sun, nutrients and water with commercial rice (Chin *et al*, 2007). Weedy rice is genetically related to cultivated rice (Gealy *et al,* 2003). Because of the genetic similarity they shared, controlling weedy rice is very difficult. Most weedy rice have the same common trait such as taller plants, fewer tillers, easy shattering of the seeds, and earlier time of flowering (Chin *et al*, 2007). The ease of shattering and seed dormancy of weedy rice can effect seed bank (Burgos *et al* 2011) and increase managerial problem in the years to come. Compared to weedy rice, cultivated rice is less efficient in terms of N absorption efficiency while weedy rice can produce more biomass per one unit of N absorbed (Burgos *et al,* 2006). During harvesting, grain taken from weedy rice can reduce the quality of milled rice due to the extra milling done to remove the red pigments from red rice seeds (Shivrain *et al,* 2008).

The Clearfield® rice was developed specifically to control weeds in the rice fields. It is a type of naturally genetically modified rice that is resistant to imidazolinone based herbicides (Croughan, 2003). In Malaysia two varieties were released, MR220-CL1 and MR220-CL2. Although this technique is effective in controlling weedy rice it also has drawbacks. As Clearfield® rice and weedy rice are genetically related, these rice have the possibility to hybridize. Natural hybridization can occur depending on the factors such as genetic and environments. In terms of genetics, weedy rice and common cultivated rice are distinctly related. Although rice is self-pollinated, rice pollen can travel long distances from their mother plant. This can cause gene flow to occur. Gene flow occurrences are very low, less than 1% or less than 200 plants per hectare but the statistics can change due to type of weedy rice and cultivated rice that are within the vicinity of the area (Shivrain *et al,* 2008).

The main concern of this hybridization is the production of progenies that have the same resistance as Clearfield® rice and thus will cause a problem for rice production. Countries like Brazil (Roso *et al*, 2010), Greece (Kaloumenos *et al*, 2013), and United State (Shivrain *et al*, 2008) all have reported gene flow from Clearfield® rice to weedy rice under field condition. For our country that also uses Clearfield® rice, the chances of gene flow are almost certain. There are three objectives for this study. The objectives of this study are:

1. To determine the morphology differences of common weedy rice with Clearfield® rice

2. To study the possibility of hybridization between Clearfield® rice and weedy rice in field condition using genetic markers as confirmation.
3. To determine the distance factor that increases the hybridization frequency between Clearfield® rice with weedy rice

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